Three decades of research on radiation-induced DNA lesions

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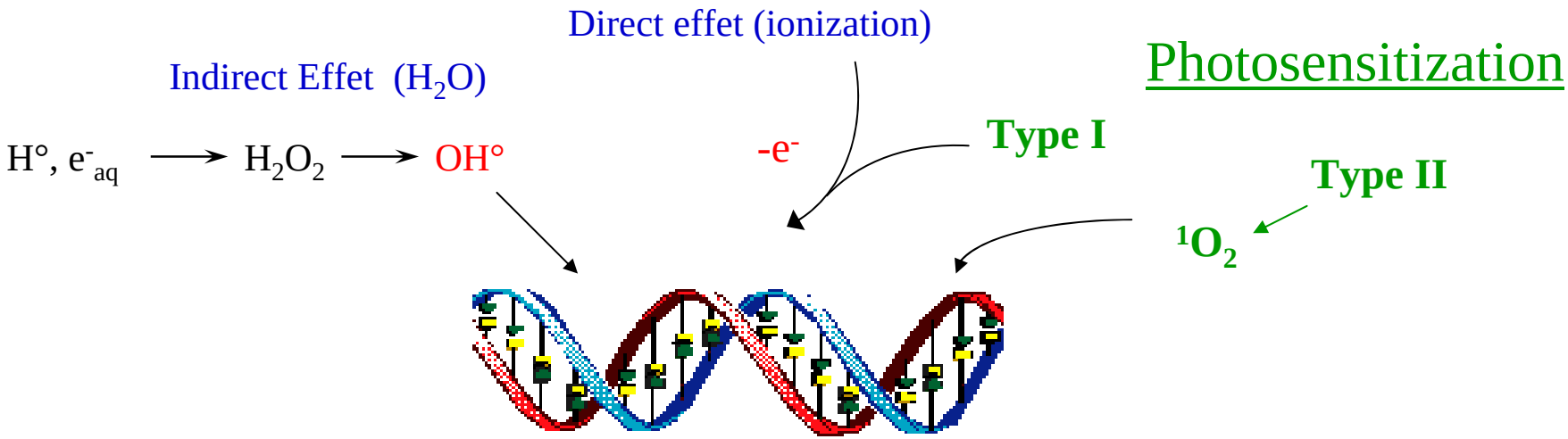
Effects of radiations on DNA



DNA damages

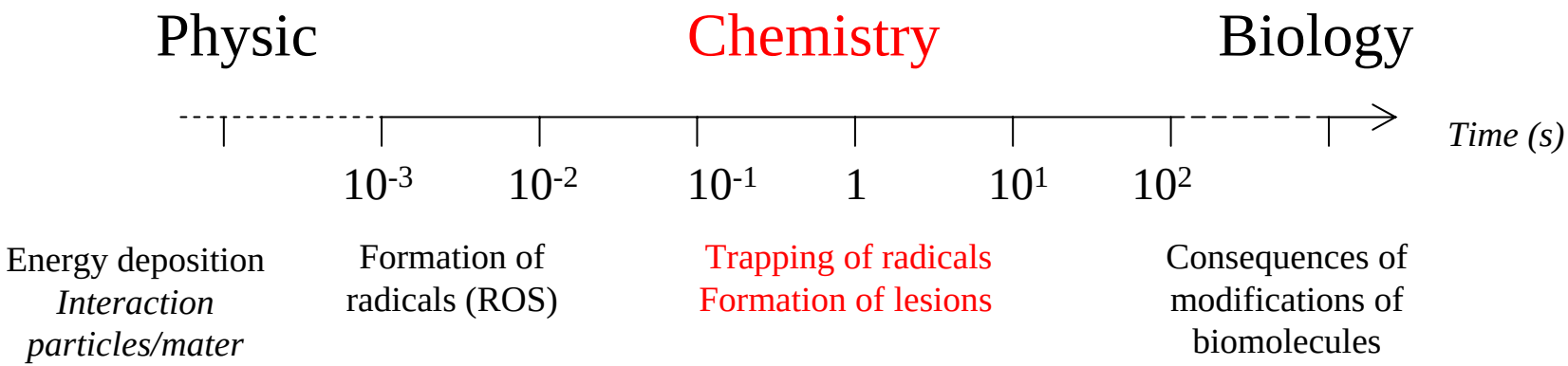
Identification of DNA lesions

Quantification in cellular DNA

Biological consequences of these lesions

Radiobiology

From physic to biology, through chemistry…



Biological consequences are directly related to chemical events…

Lesions generated in cellular DNA ?
What are their yields of formation ?
Kinetic & fidelity of repair, mutagenicity…

DNA damage

DNA
Support of genetic information

Genotoxic agents

Lesions

Mutations

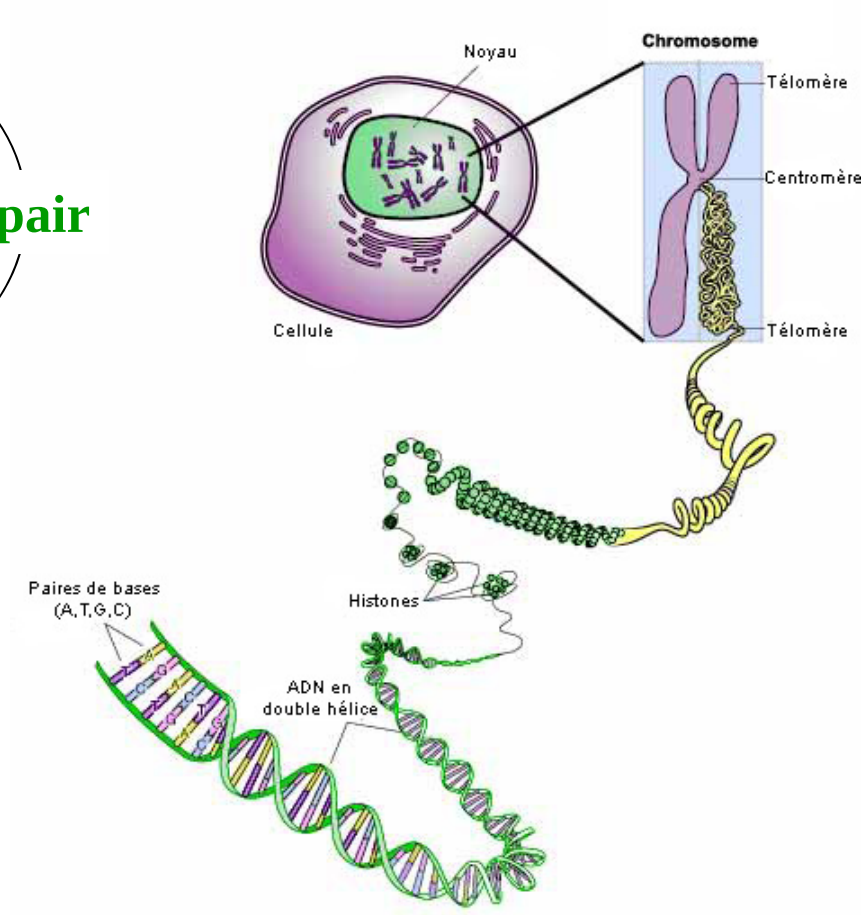
Cancer

Lethality

Aging

Evolution

Repair



What are the chemical modification induced by radiation?

Main approaches used to identify DNA lesions

Studies using model systems exposed to radiation

Nucleosides (protected or not)
Nucleotides, short oligonucleotides

Isolation and Identification of the decomposition products

HPLC
NMR (^{1}H, ^{13}C, ^{15}N, 1D et 2D)
Mass spectrometry …

Mechanism of formation

Different experimental conditions
Times resolved spectroscopies
Labeling experiments ($H_2{}^{18}O$, $^{18}O_2$)

Detection of the lesions in isolated DNA, in cells

HPLC-Fluorescence, Amperometry
HPLC-MS/MS

Today about 70 different radiation-induced DNA lesions have been identified
About 15 are detected in cellular DNA

Importance of chemistry…

In the chemistry laboratory (Warsaw Museum of Industry and Agriculture) **Maria completed a systematic course of chemical, qualitative and quantitative analysis**… Maria sklodowska left the laboratory **with enormous chemical knowledge and analytical skills**, which were to help her in her later research work in Paris.

Beginning of twentieth century Maria said after a lecture in Warsaw:

"If Professor Napoleon Milicer and his assistant Kossakowski had not taught me analysis so well in Warsaw, I would never have been able to isolate radium"

From "Maria Sklodowska-Curie and Radioactivity"
By Josef Hurwic, *Galan Edition, Warsaw, 2011*

Among other things, the Curies found that rays emitted by radium can transform oxygen into ozone, thus they gave **radiation chemistry** its beginning

P. Curie, M. Curie, "Effets chimiques produits par les rayons de Becquerels" ibid. (1902) 129, 823-825

Importance and role of **Chemistry** in Radiation-biology

If the harmful effect of radiations are known from a long time, identification of the produced DNA lesions is relatively recent

1902-First cases of radiation induced skin cancer reported
1970's 1980's - Radiation chemistry in the field of DNA damage

Hariharan PV, Cerutti PA (1972) Formation and repair of gamma-ray induced thymine damage in Micrococcus radiodurans. J Mol Biol 66: 65–81.

Schellenberg KA, Shaeffer J, Nichols RK, Gates D (1981) Characterization of radiation damage to DNA by reaction with borohydride. Nucleic Acids Res 9: 3863–3872.

Cerutti PA. (1974) Effects of ionizing radiation on mammalian cells. Naturwissenschaften 61: 51–59.

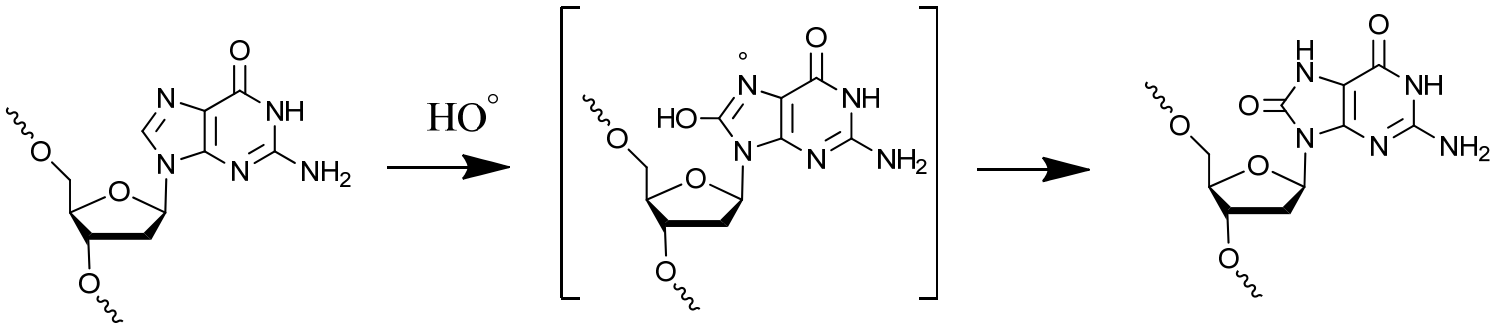
Téoule R, Bonicel A, Bert C, Cadet J, Polverelli M (1974) Identification of radioproducts resulting from the breakage of thymine moiety by gamma irradiation of E coli DNA in an aerated aqueous solution. Radiat Res 57: 46–58.

Hariharan PV, Cerutti PA (1977) Formation of products of the 5,6-dihydroxydihydrothymine type by ultraviolet light in HeLa cells. Biochemistry 16: 2791–2795.

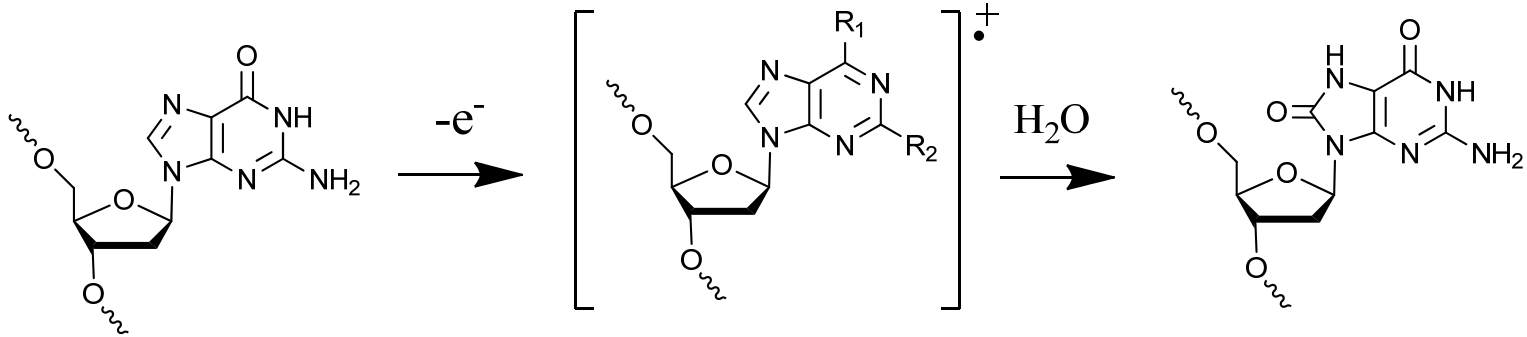
Kasai, H. and Nishimura, S., Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agent. (1984) *Nucleic Acids Res.*, **12**, 2137-2145.

Kasai, H., Tanooka, H. and Nishimura, S., Formation of 8-hydroxyguanine residues in DNA by X-irradiation. (1984) *Gann.*, **75**, 1037-1039.

Radiation chemistry: Mechanism of formation of 8-oxodGuo



Kasai, H. *et al.*, *Nucleic Acids Res.* **12**, (1984). 2137-2145
Addition of HO° at C8 of dGuo (indirect effect)

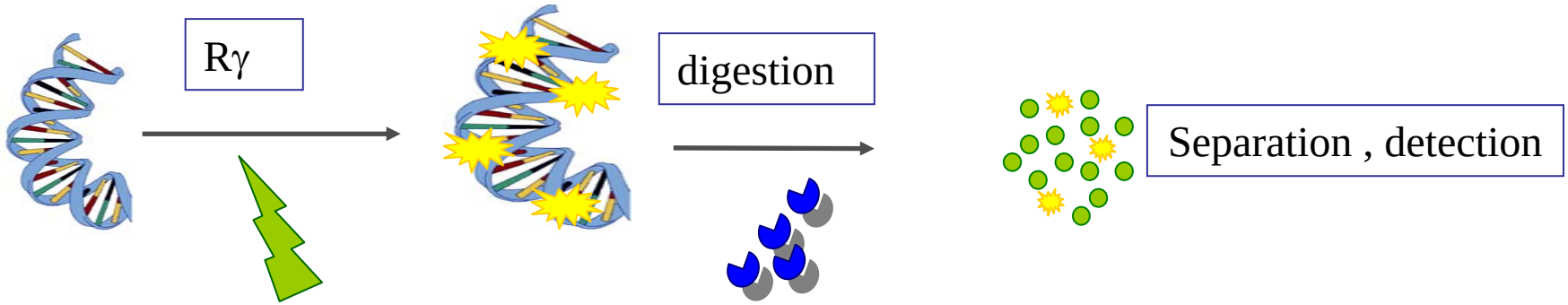


Kasai, H. *et al.*, *J. Am. Chem. Soc.* **114**, (1992). 9692-9694
Hydration of guanine radical cation (direct effect)

Importance of **Analytical** Chemistry

Measuring DNA lesions in cells is a challenging analytical problem.

Experimental approach used:



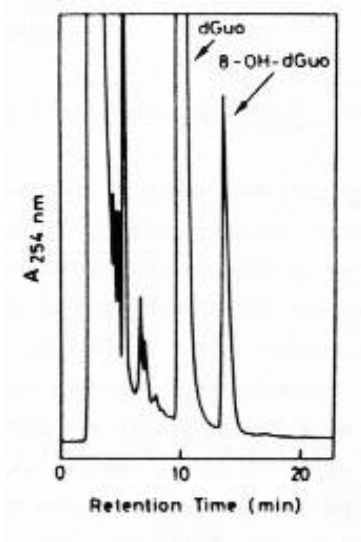
When cells are irradiated, DNA is first isolated from other cellular constituents

The method used for detection should be highly sensitive to detect **a few modification per million normal nucleosides**.

Detection of 8-oxodGuo : a long story…

-First reports: HPLC with UV detection

Detection not very sensitive, not specific
Not sensitive enough for DNA



Kasai et al. 1984

-**Development of HPLC-EC detection**

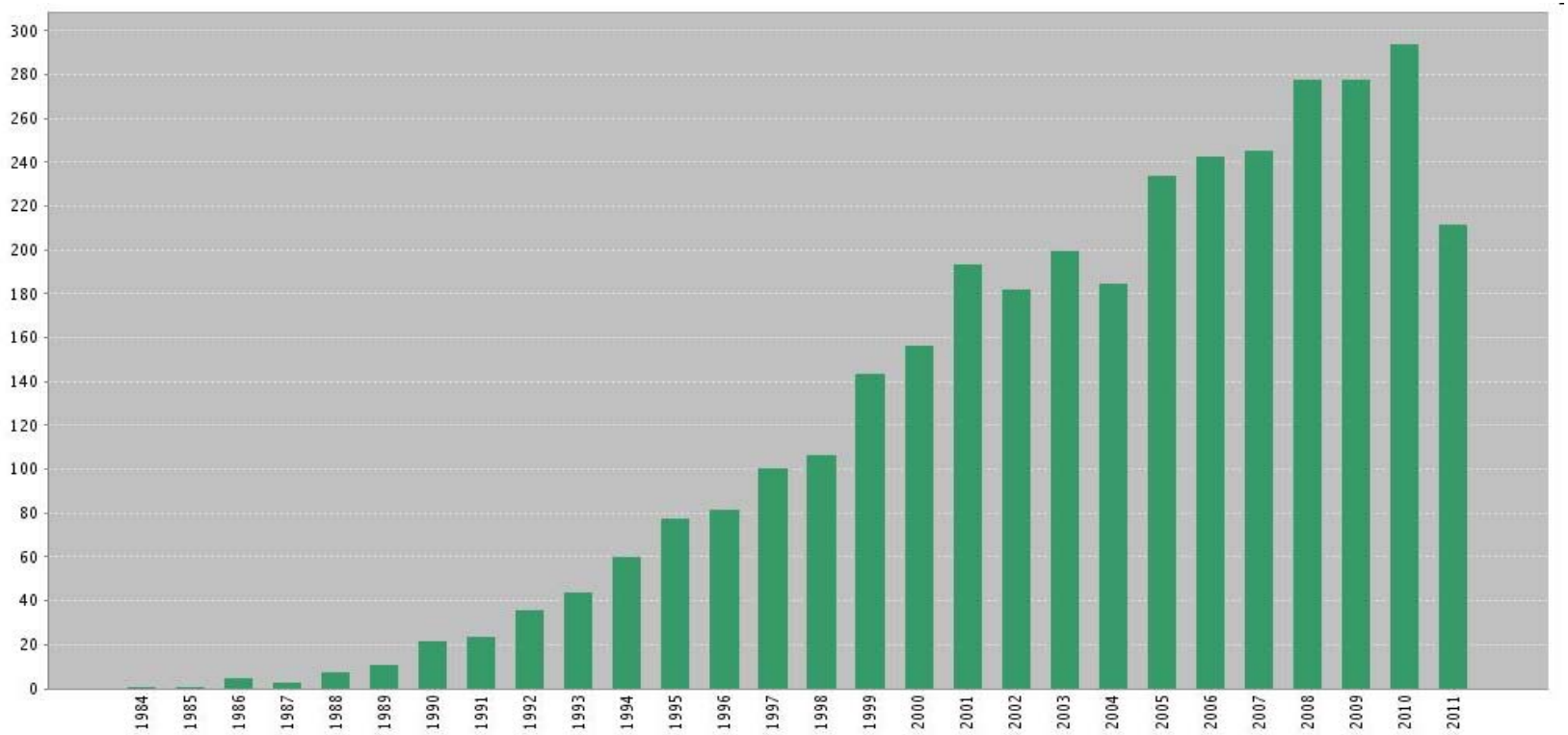
Floyd, R. A. *et al.*, *Free Radic. Res. Commun.* **1**, (1986). 163-172

The product is oxidized at a defined potential and produced electrons are detected
Highly sensitive, and specific (normal nucleosides are not detected)

The sensitive (and facility of use) of that method is probably at the origin of the **popularity of 8-oxodGuo**.

8-oxodGuo, a well-studied DNA lesion

Number of publications per year (*web of knowledge*)



Total 5853 keywords 8-hydroxy-2'-deoxyguanosine or 8-oxo-7,8-dihydro-2'-deoxyguanosine

Gas-chromatography mass spectrometry (GC-MS)

Dizdaroglu, M. (1984) *J Chromatogr*, **295**, 103-121.

Since DNA bases are not volatile, they have to be derivatized (introduction of TMS groups) prior to the measurement

But…

Halliwell, B. and Dizdaroglu, M., Commentary. The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. (1992) *Free Radic. Res. Commun.*, **16**, 75-87.

... To date, fewer studies upon DNA fresly-isolated from cells and tissues have been done with GC/MS-SIM than with HPLC, but the figures available show around 40 8-OHGua per 10^6 DNA bases, about 2- to 11-fold greater than the figures recorded by HPLC...

Which method is the correct one ?

The derivatization reaction induces oxidation of normal bases and thus, GC-MS **overestimates** the level of 8-oxodGuo in DNA by up to 3 orders of magnitudes !

Reprinted from Chemical Research in Toxicology, 1995, *8*.

Determination of 8-Oxoguanine in DNA by Gas Chromatography−Mass Spectrometry and HPLC−Electrochemical Detection: Overestimation of the Background Level of the Oxidized Base by the Gas Chromatography−Mass Spectrometry Assay

Jean-Luc Ravanat,† Robert J. Turesky,*,† Eric Gremaud,† Laura J. Trudel,‡ and Richard H. Stadler†

Nestec Ltd., Nestlé Research Centre, Vers-Chez-Les-Blanc, 1000 Lausanne 26, Switzerland, and Division of Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received April 21, 1995®

This have highlighted another potential problem, DNA oxidation could also occur during extraction and digestion…

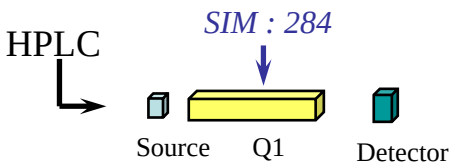
*Ravanat, J.-L. et al., Carcinogenesis **23**, (2002). 1911-1918.*

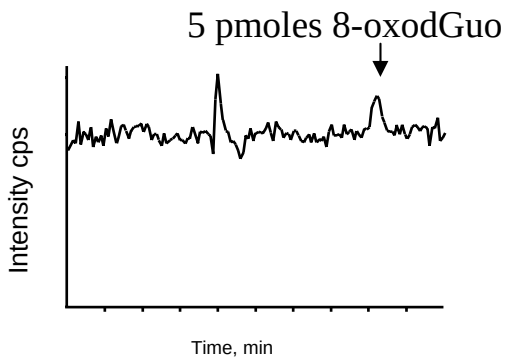
Development of HPLC coupled to tandem mass spectrometry

Electrospray ionisation developed in mid-80s by Dr John B. Fenn Nobel Laureate in Chemistry in 2002

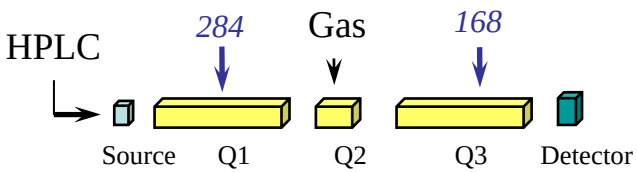
Ravanat, J.-L., et al. (1998) *J. Chromatogr. B*, **715**, 349-356.

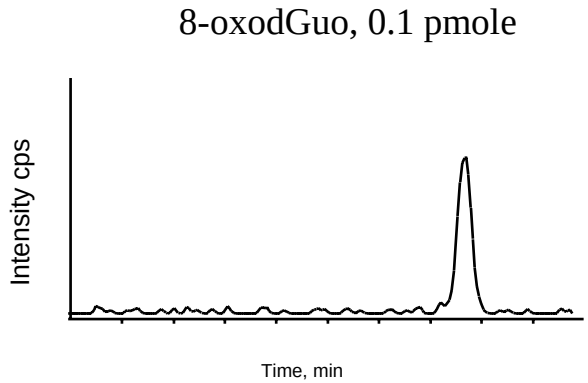
HPLC-MS (SIM)





HPLC-MS/MS (MRM)

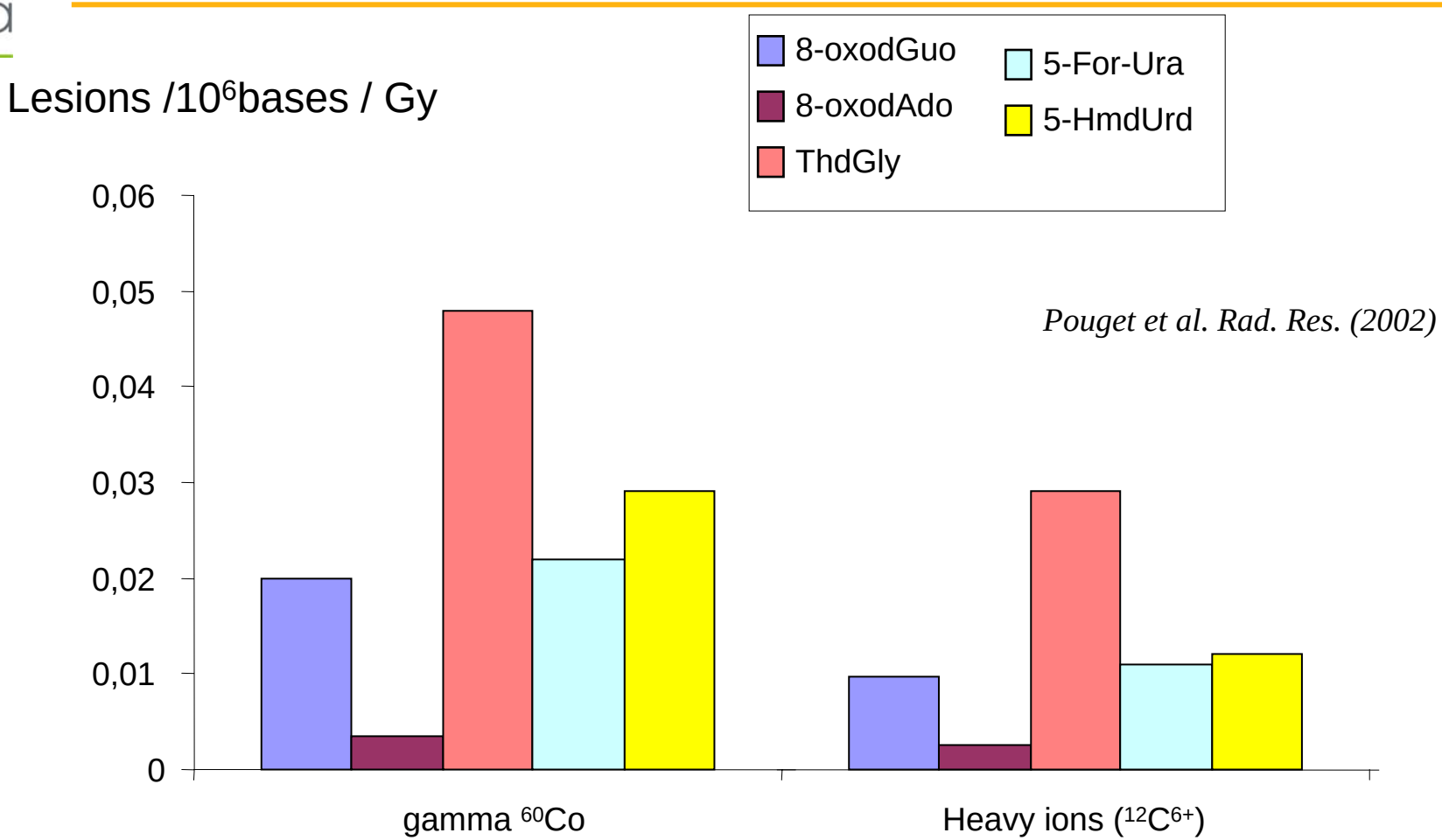




Tandem mass spectrometry is required to obtain a sensitivity compatible with the level of lesion measured in cells

Simultaneous determination of several radiation-induced DNA lesions

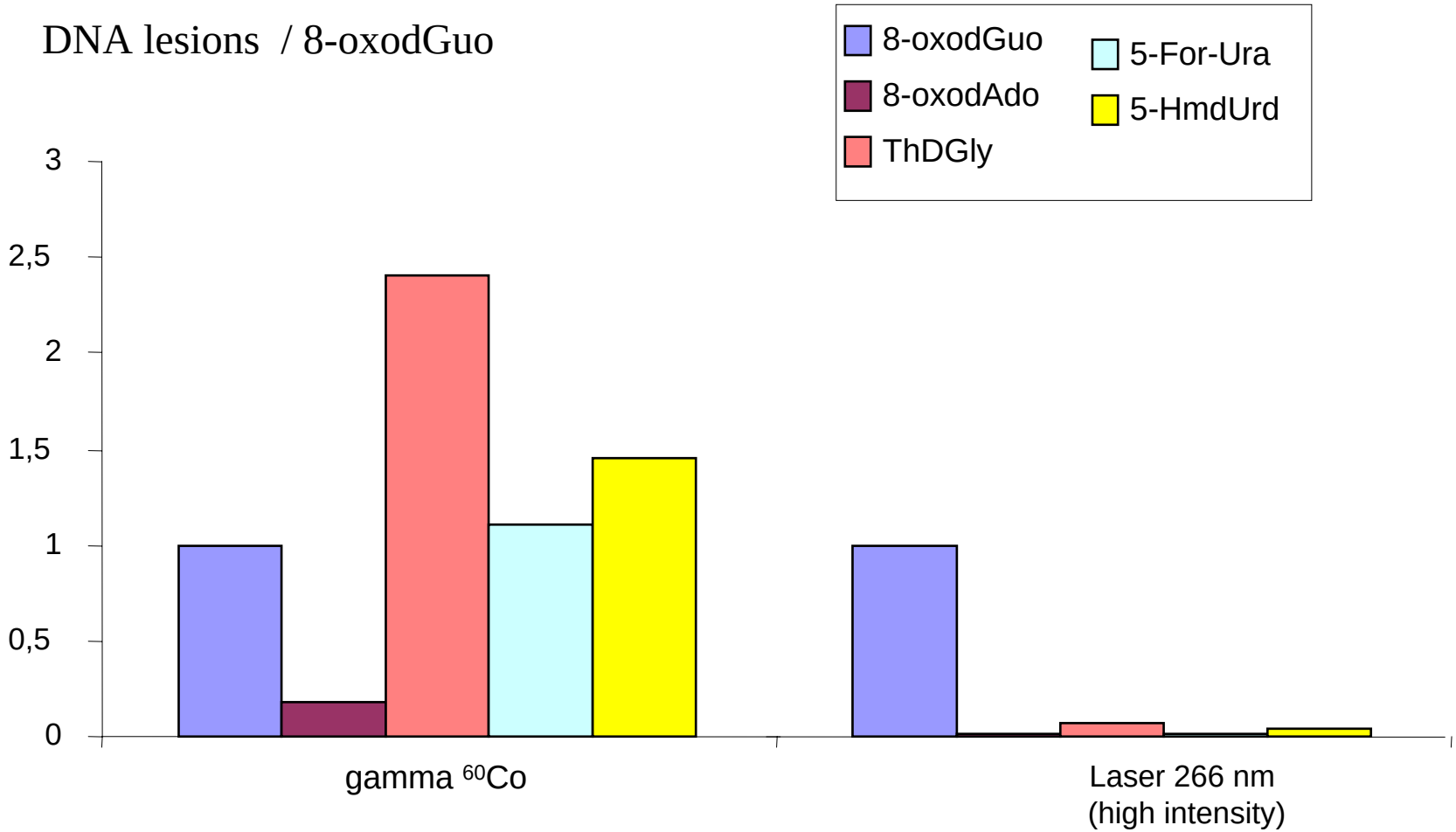
Formation of lesions the DNA of irradiated cells



Pouget et al. Rad. Res. (2002)

Yield of formation of damage is low!
8-oxodGuo is not the major lesion produced in cells

Direct *versus* indirect effect ?



The indirect effect (role of HO°) is the main mechanism.

Douki, T. *et al.*, *Int. J. Radiat. Biol.* **82**, (2006). 119-127

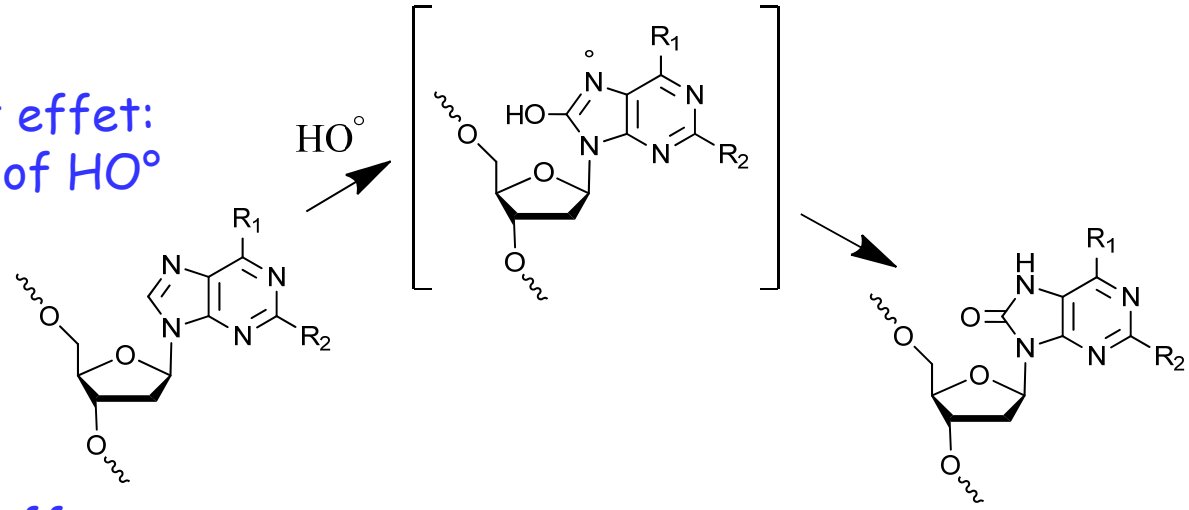
Observation:

Reactivity of DNA bases in dsDNA is at least partly different to that determined at the nucleoside level !

Study of chemical reactions in dsDNA

Formation of 8-oxopurines in dsDNA

Mechanisms of radiation-induced formation of 8-oxodGuo and 8-oxodAdo



The indirect effect is predominant (role of HO°)

Pending questions :

-Different reactivity between free nucleosides and dsDNA !

Ravanat, J.-L. *et al.*, *J. Am. Chem. Soc.* **125**, (2003). 2030-2031

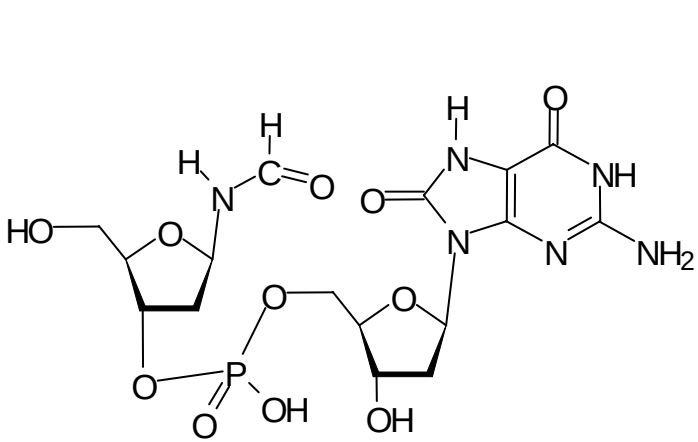
-Why in dsDNA 8-oxodGuo >> 8-oxodAdo?

Is there (at least) another mechanism involved ?

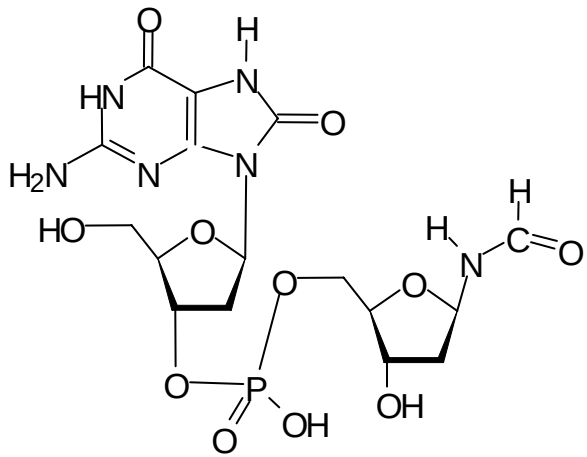
Radiation-induced formation of tandem lesions

Tandem lesion Formylamine(dF)/8-oxodGuo

Bourdat, A.-G., *et al.* (2000) *J. Am. Chem. Soc.*, **122**, 4549-4556.



dF/8-oxodGuo



8-oxodGuo/dF

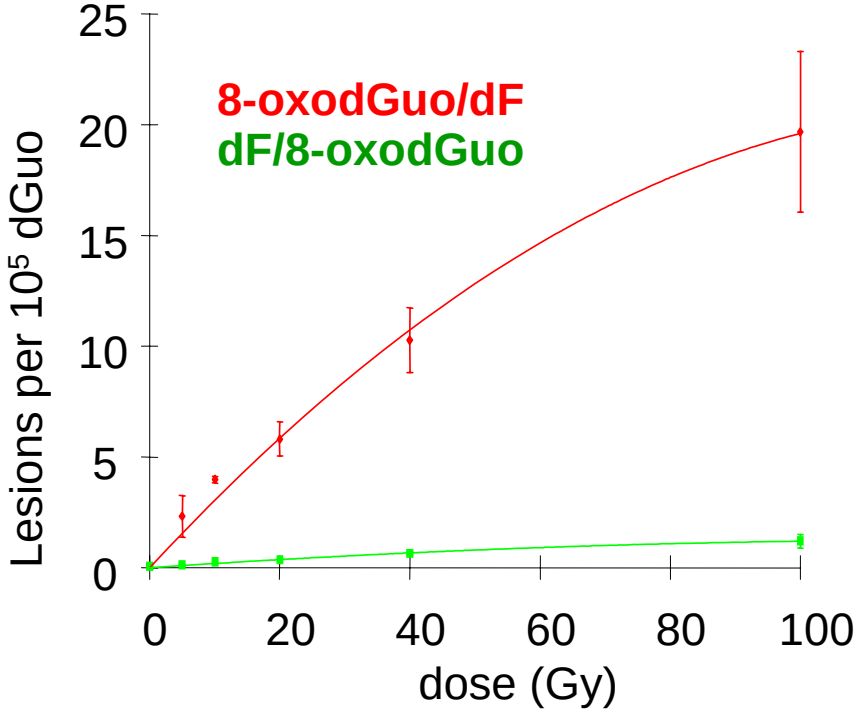
Efficacy of repair of these tandem lesions by BER?

Are these lesions generated in irradiated dsDNA ?

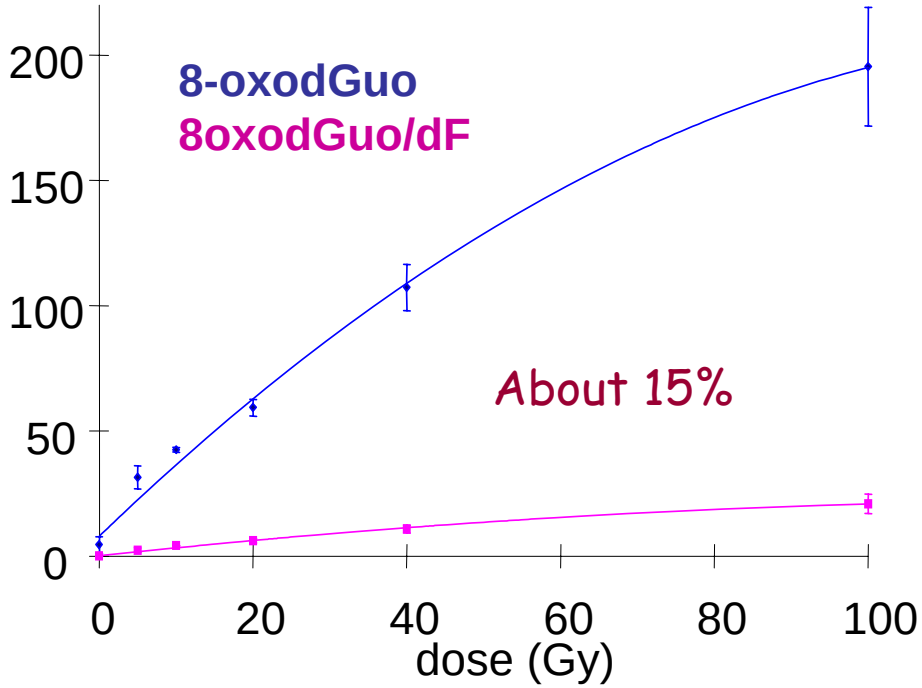
Radiation-induced formation of tandem lesions

Bourdat, A.-G., *et al.* (2000) *J. Am. Chem. Soc.*, **122**, 4549-4556.

Linearity of formation
Sequence effect

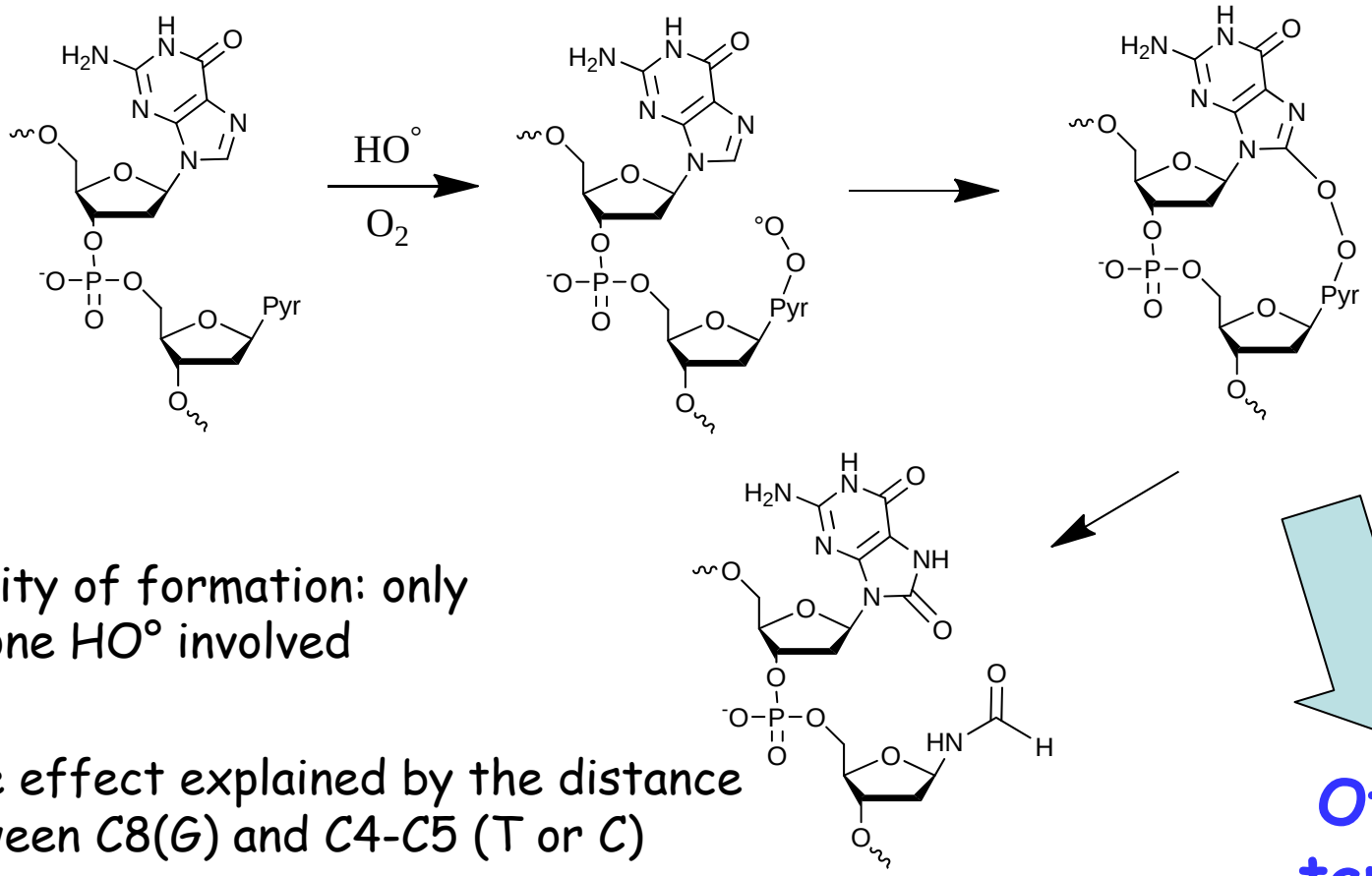


Importance/8-oxodGuo



Mechanism of formation ? Only one radical involved ?

Mechanism of formation of tandem lesions



Linearity of formation: only one HO° involved

Sequence effect explained by the distance between C8(G) and C4-C5 (T or C)

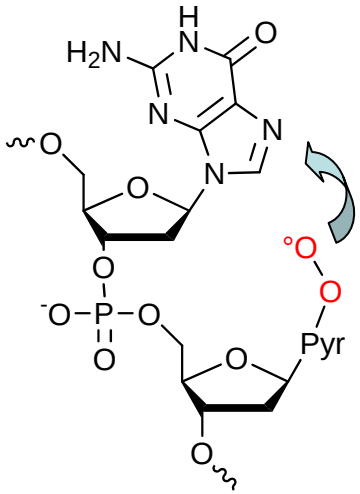
Douki, T., et al. (2002) *Chem. Res. Toxicol.*, **15**, 445-454.

8-oxodGuo/dF

Other tandem lesions ?

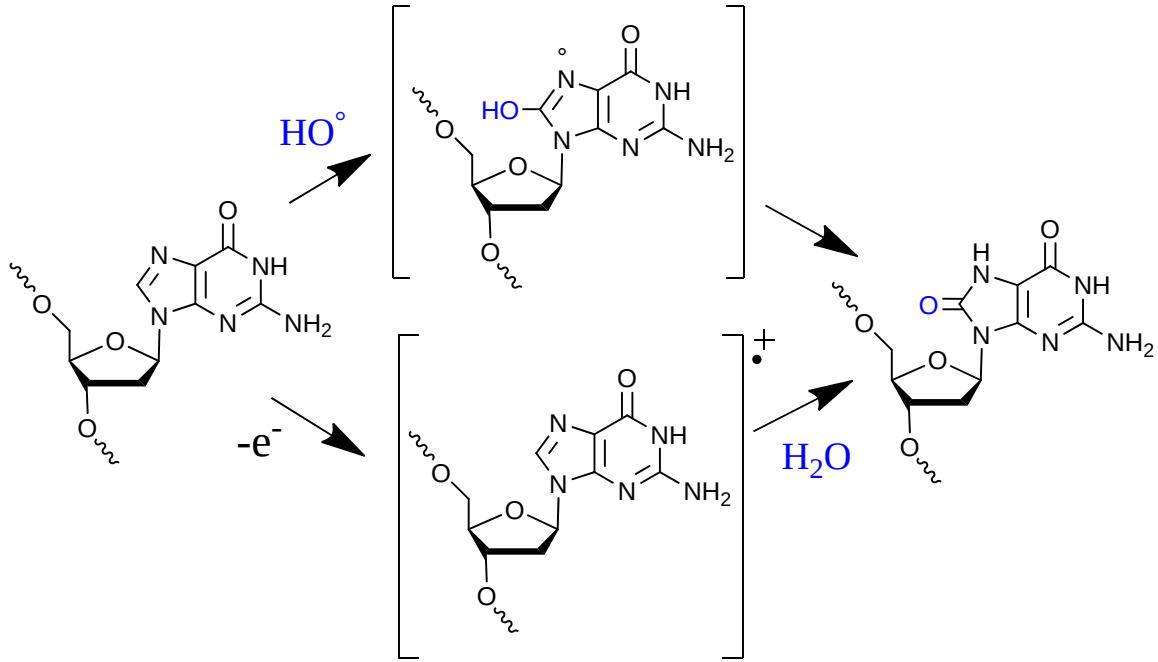
What is the importance of such a reaction ?

Proportion of 8-oxopurine involved in tandem lesions?



If a peroxyl radical is involved (tandem DNA lesions) the oxygen atom incorporated in 8-oxodGuo comes from molecular oxygen

If not, for both direct and indirect effects, incorporated oxygen atom comes from water



Proportion of 8-oxopurine involved in tandem lesions?

Determination of the relative importance of tandem lesions

Origin of the oxygen atom?

DNA irradiation performed in the presence of either

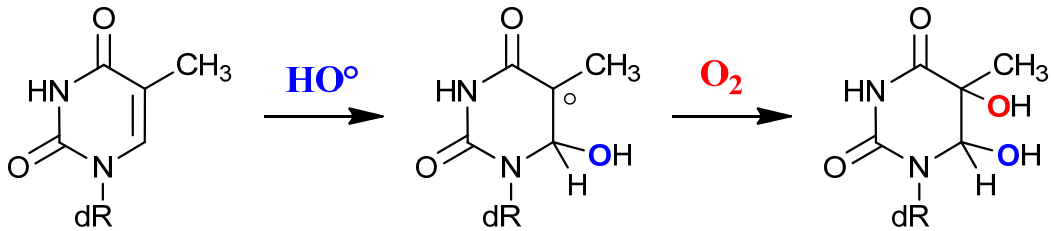
- $H_2{}^{18}O$
- ${}^{18}O_2$

DNA digestion and HPLC-MS/MS determination of both 8-oxoPur (M) et ^{18}O-8-oxoPur (M+2)

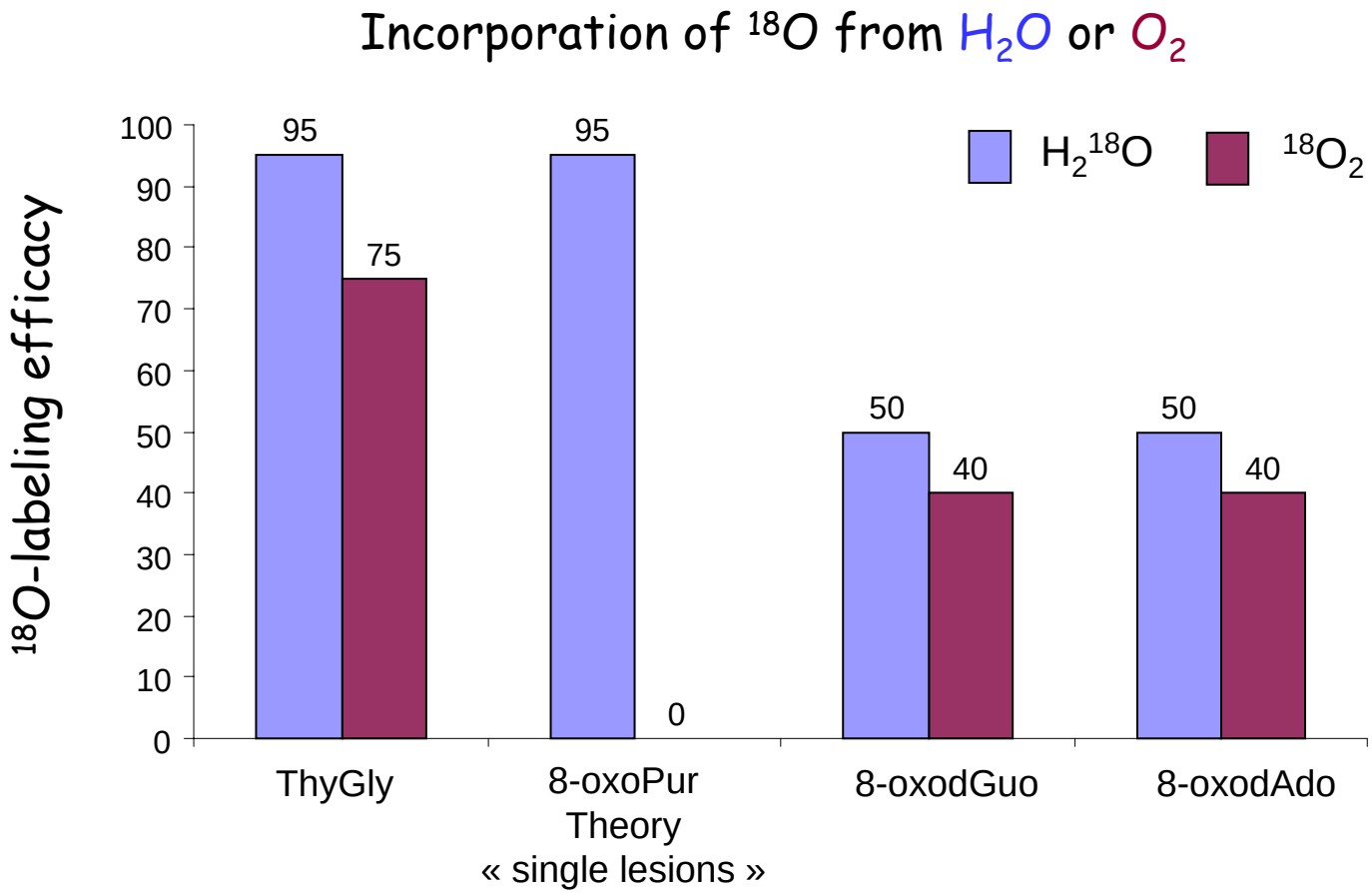
Ravanat, J.-L. (2000) *J. Biol. Chem.* **275**, 40601-40604

Origin of the oxygen atom and quantification of oxidized purine bases

Use of thymidine glycols as an internal standard (to evaluate isotopic enrichment of the solution)



Proportion of 8-oxopurines involved in tandem lesions



For 50% of 8-oxopur (both 8-oxodGuo and 8-oxodAdo) the oxygen atom comes from molecular oxygen!

Formation of tandem lesions induced by peroxyl radicals

Use of polynucleotides

Poly(dA)-poly(dT)

…TpTpTpTpTpTpTpTpT…
…ApApApApApApApApA…

Poly(dG)-poly(dC)

…CpCpCpCpCpCpCpCpC…
…GpGpGpGpGpGpGpGpG…

Poly(dA-dT)

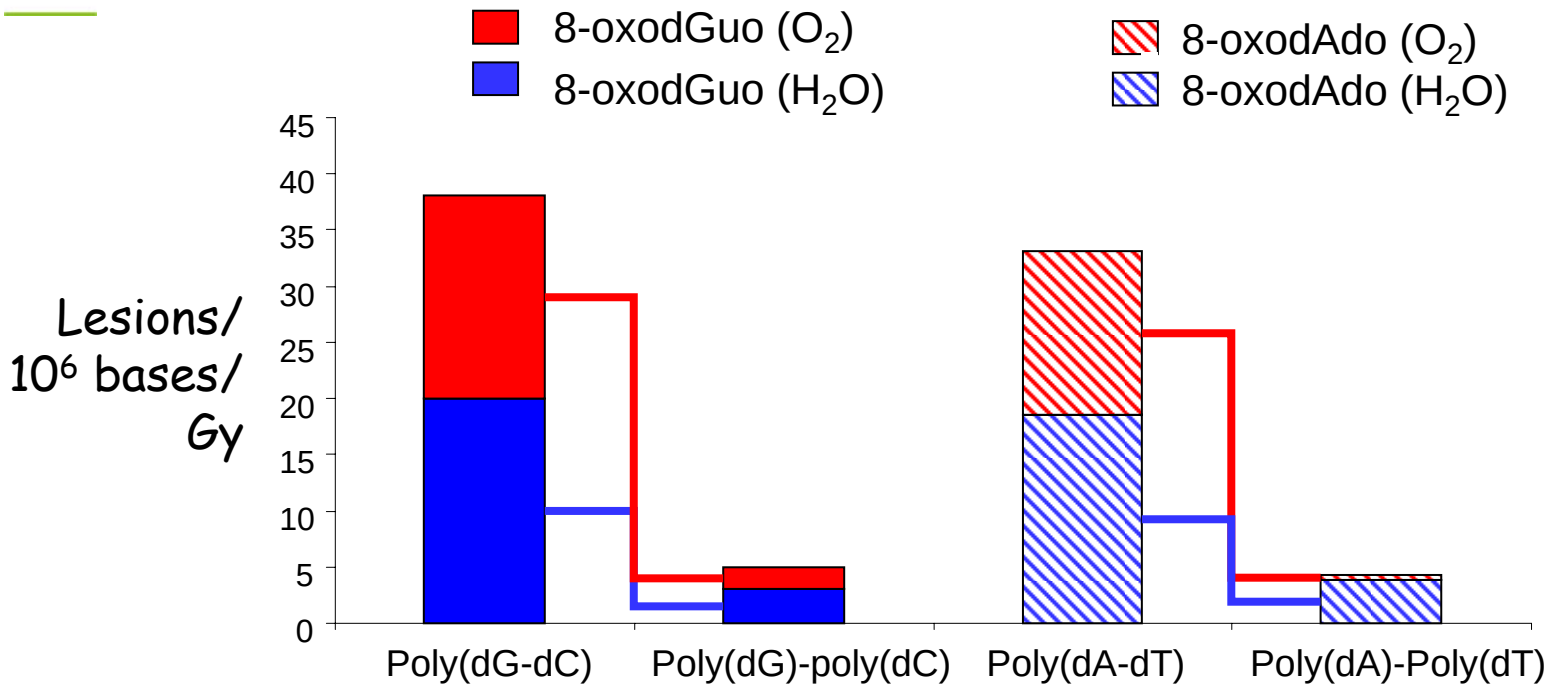
…TpApTpApTpApTpApT…
…ApTpApTpApTpApTpA…

Poly(dG-dC)

…CpGpCpGpCpGpCpCpC…
…GpGpGpGpGpGpGpGpG…

Always double stranded DNA
Same concentration of nucleotides (G and A in particular)
Only relative position between purine & pyrimidine bases is different

Quantification of lesions produced by γ-irradiation



Purine opposite pyrimidine

- almost no tandem DNA lesions (almost no incorporation of O from O_2)
- Peroxyl radicals do not react with bases on the opposite strand

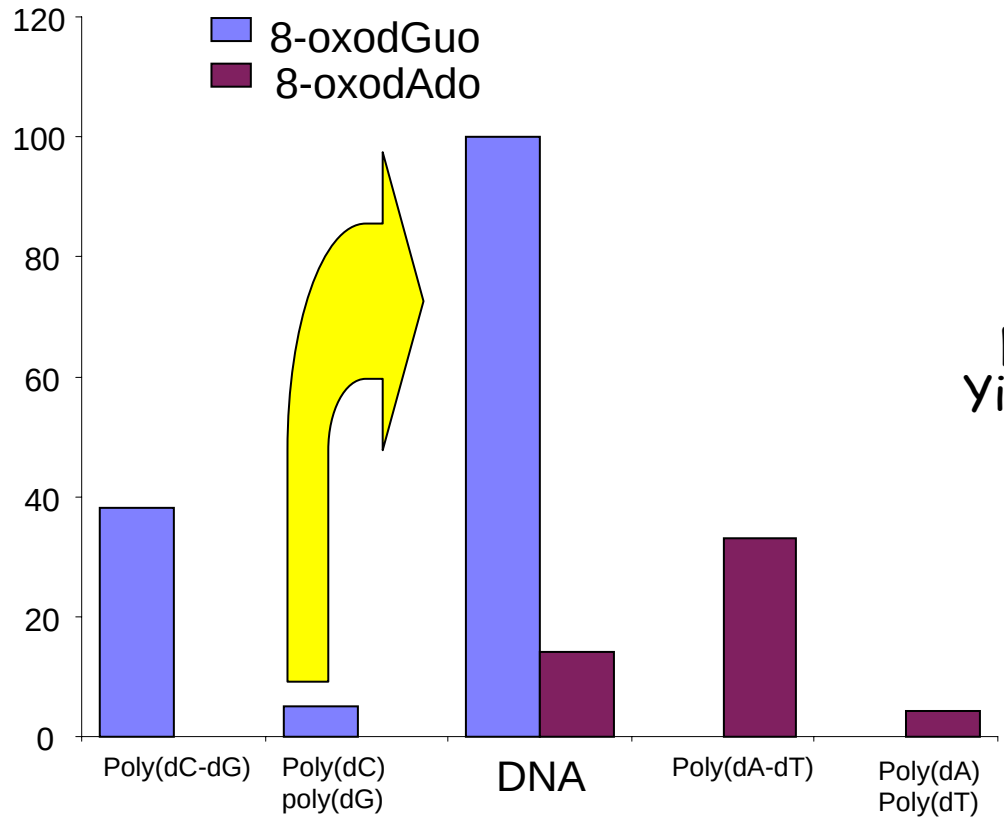
Alternation purine/pyrimidine on the same strand

-Formation of 8-oxoPur (O_2) = ROO° addition (as for 8-oxodGuo/dF)

-Increase yield of 8-oxoPur (H_2O) : ROO° inducing e^- transfer ?

Same amounts of HO° and purines but different numbers of lesions !

Quantification of lesions produced by γ-irradiation



For 8-oxodGuo, compared to poly(dG)-poly(dC) Yield is increased by a factor of 20!

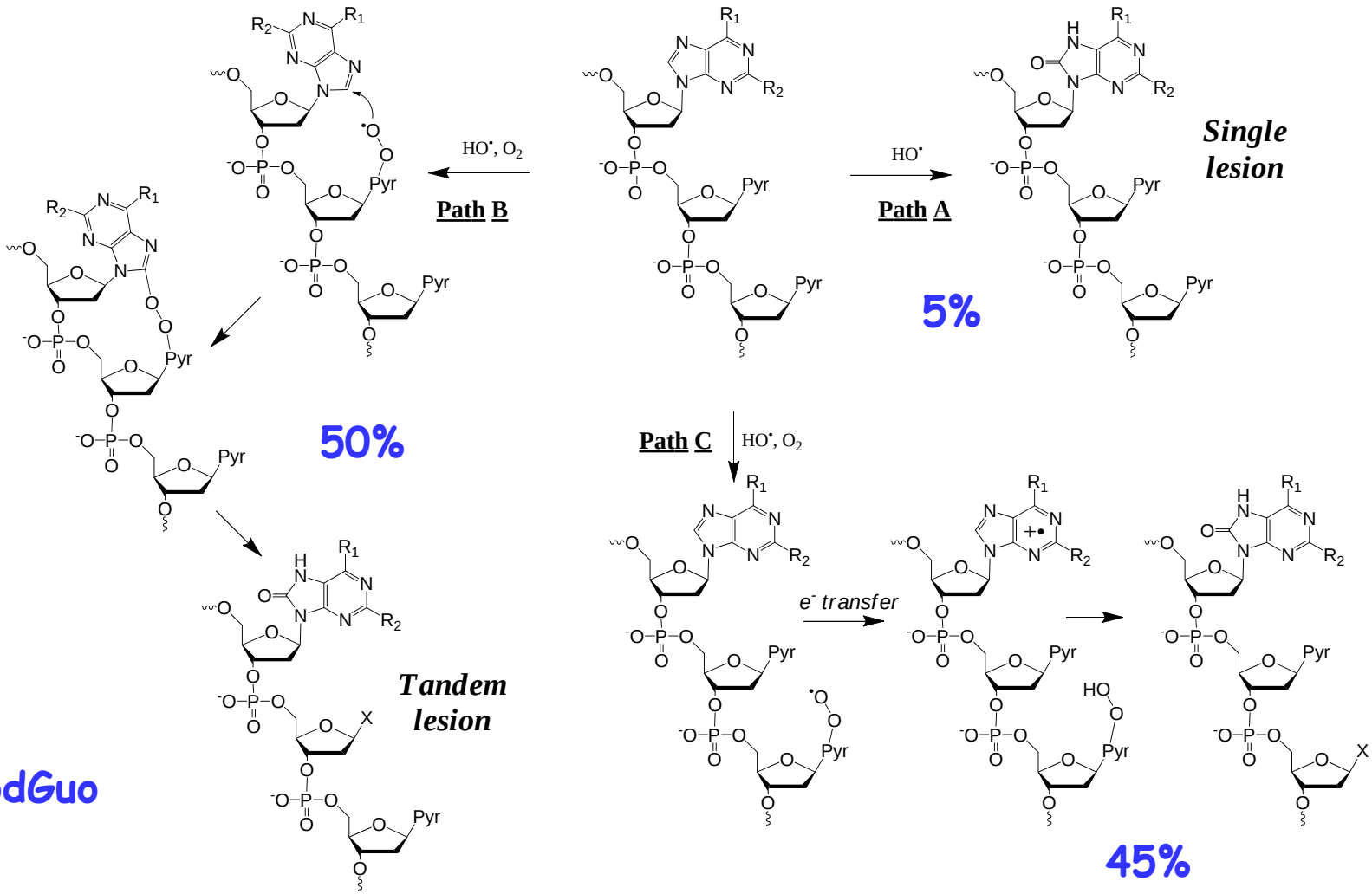
Similar yields of formation of 8-oxodGuo and 8-oxodAdo !

In dsDNA (containing the four bases)

-Decreased formation of 8-oxodAdo compared to poly(dA-dT)

-Increased formation of 8-oxodGuo : role of ROO°, e^- transfer

Mechanisms of formation of 8-oxoPur



Paths B and C are sequence dependent!

Biological importance of tandem lesions ?

A biologist would say :

"And so what ?"

Whatever the mechanism is, 8-oxodGuo is still 8-oxodGuo !

Such a lesion is mutagenic but efficiently repaired in cells (mostly by BER) !

Questions :

Is that also true for 8-oxodGuo involved in tandem lesions ?

Does the adjacent pyrimidine modification play a role ?

Are repair and mutagenicity affected by the adjacent modification?

Repair efficacy of tandem lesions

8-oxodGuo is usually excised by Fpg (E. Coli) and hOGG1 (human)

Is excision of 8-oxodGuo involved in tandem damage also efficient?

Experimental approach:

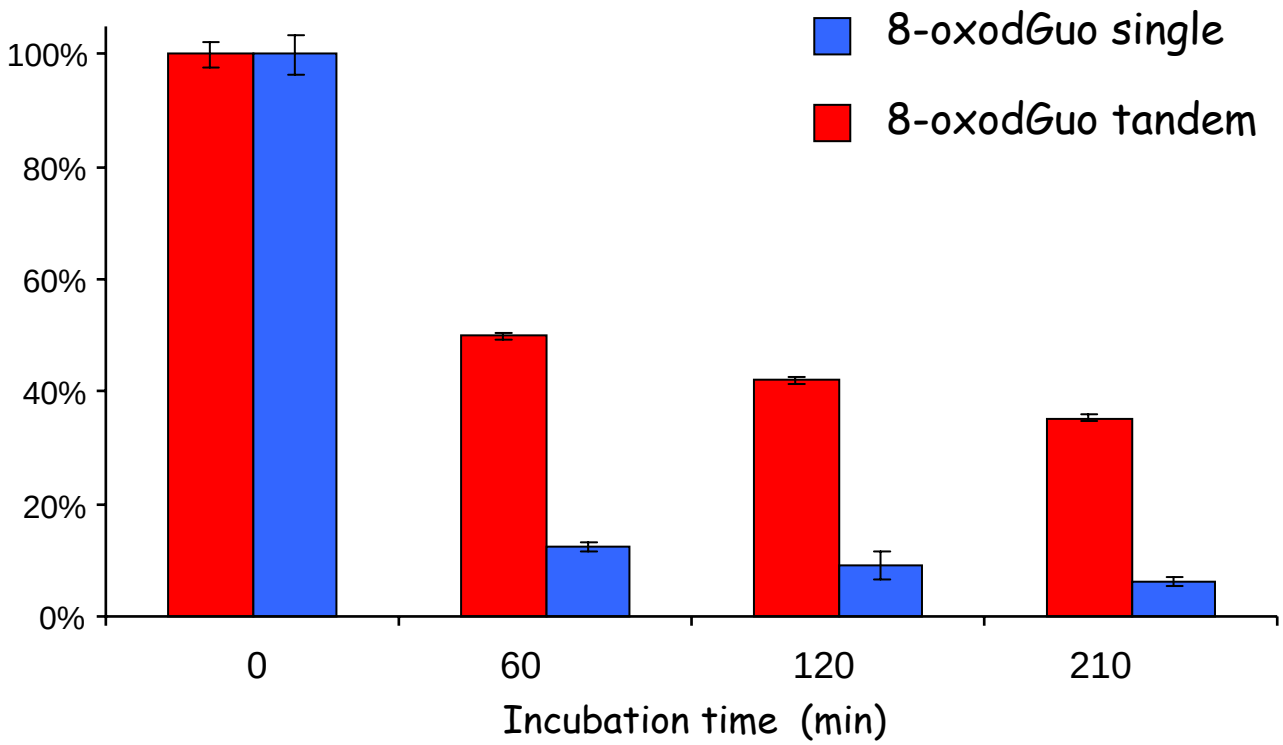
dsDNA ($H_2{}^{18}O$) → Gamma irradiation → ^{18}O-8-oxodGuo (Single) and ^{16}O-8-oxodGuo (Tandem)

Incubation with DNA glycosylases (Fpg et hOGG1)

Quantification of remaining 8-oxodGuo « single » or « tandem »

DNA Repair : Fpg (E. Coli)

Remaining levels of 8-oxodGuo (unrepaired lesions)



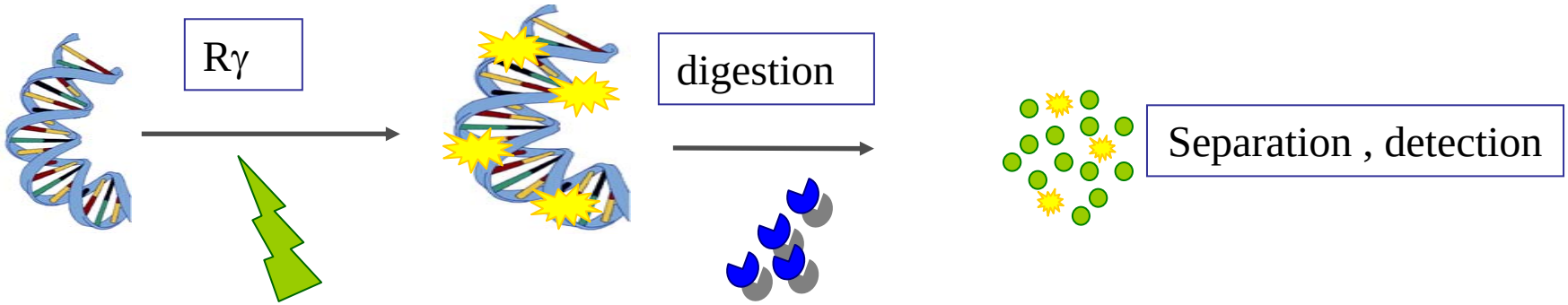
Tandem lesions are partly refractory to repair by Fpg

Coll P. Radicella

Unknown DNA lesions

Are there still unidentified DNA lesions that are significantly generated in dsDNA, and barely formed at the nucleoside level ?

Strategy : search for unknown lesions in irradiated isolated DNA



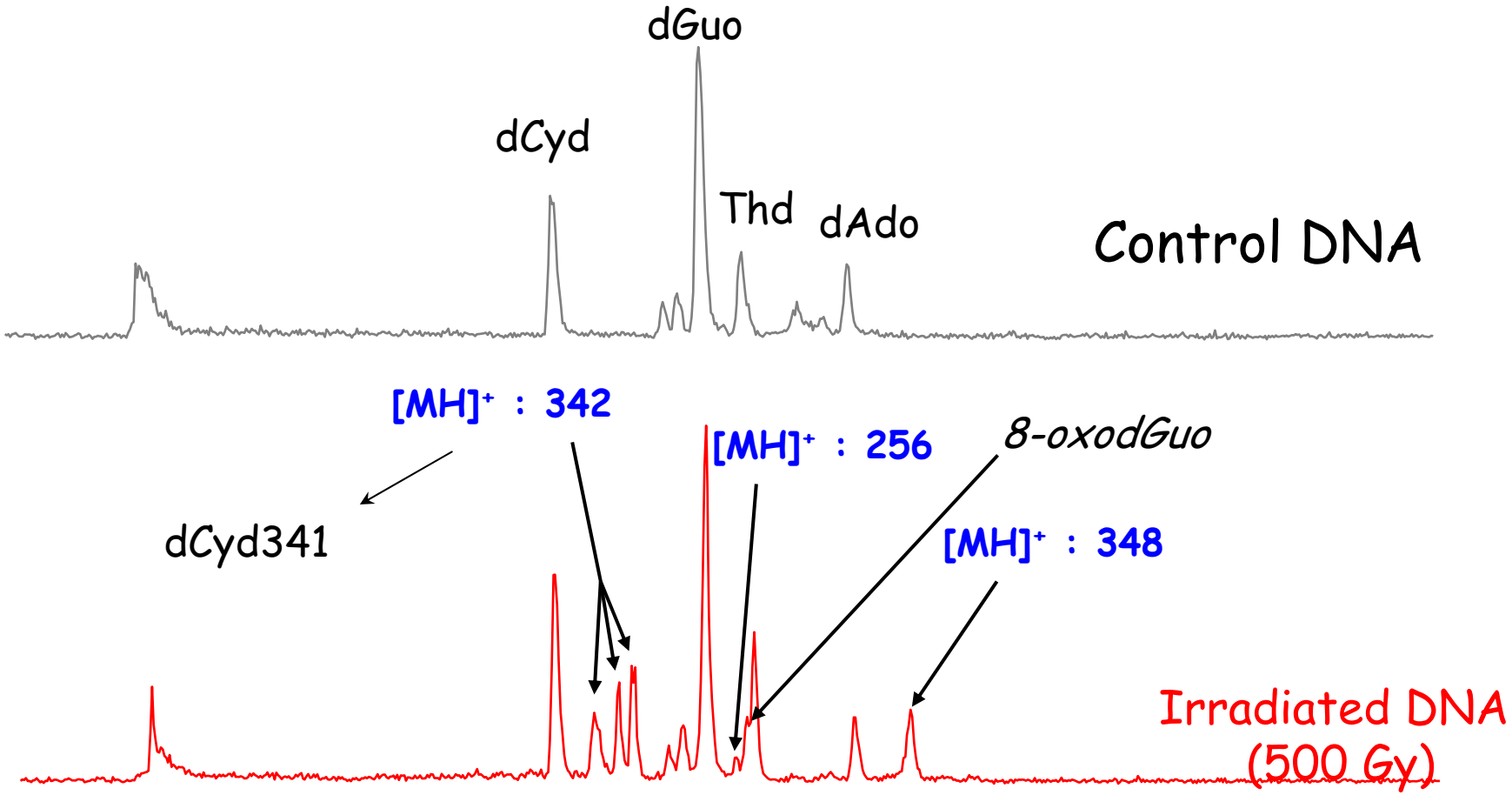
A specific and sensitive detection method is required !

To detect less than 1 unknown lesion per 1000 bases

HPLC-MS/MS: using so-called neutral loss scan mode to search for the presence of unknown nucleosides

Results…

Additional peaks should be due to the presence of DNA lesions



Is dCyd341 generated and detectable in cellular DNA ?

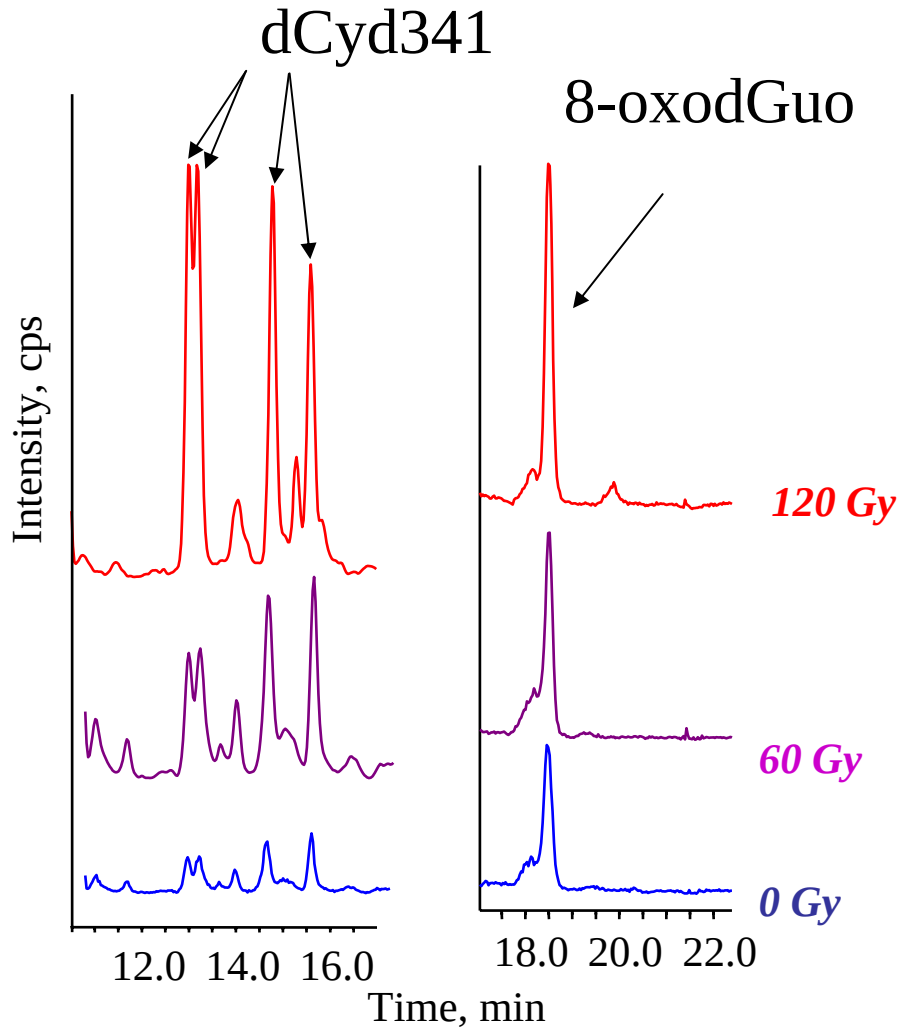
Strategy

Gamma irradiation of cells
DNA extraction*
Enzymatic digestion*
HPLC-MS/MS analysis
(MRM Mode)

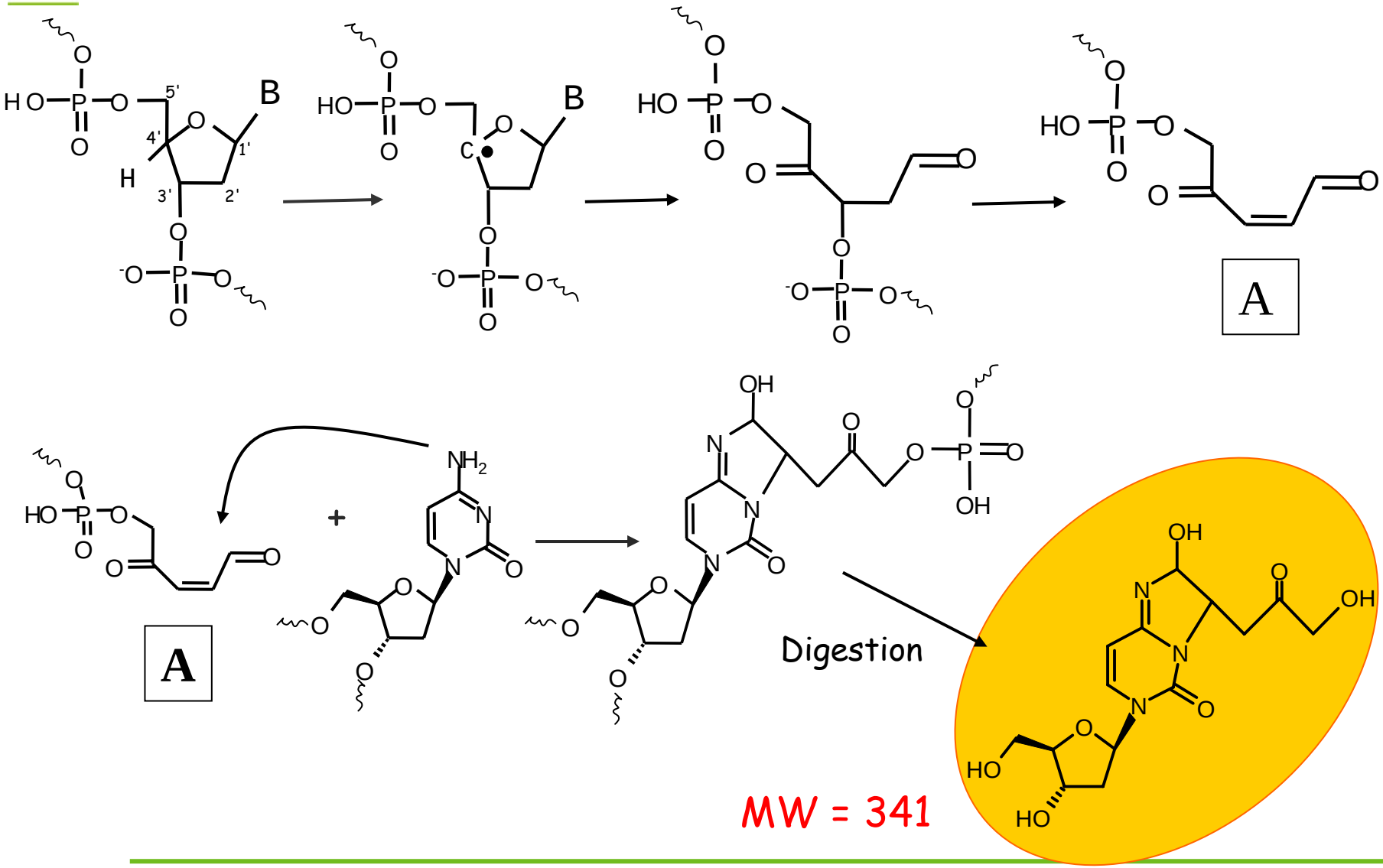
*Ravanat et al. (2002). *Carcinogenesis*, 23, 1911-1918.

Presence of dCyd341
in untreated cells
Endogenous origin
(no quantitative data)

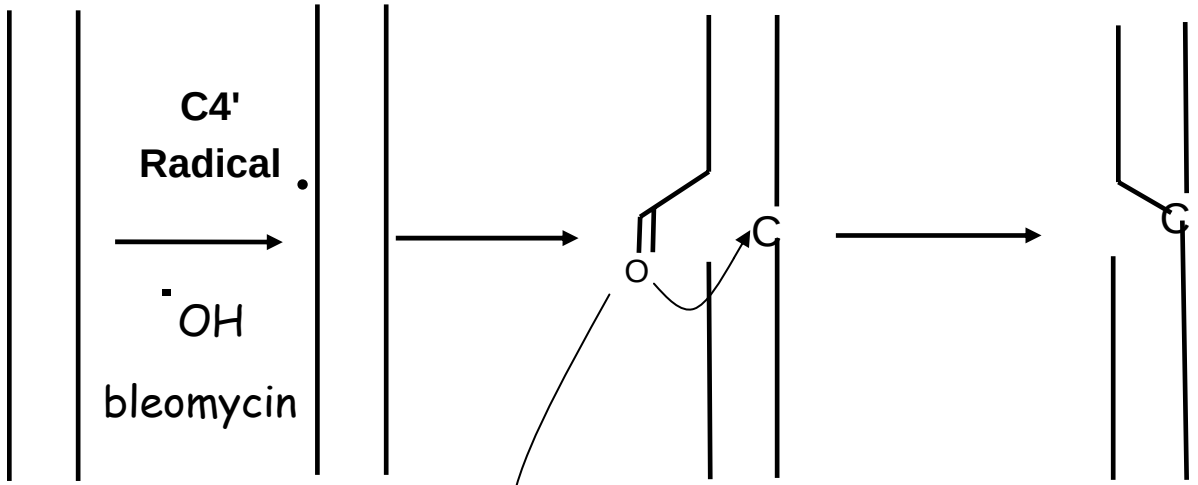
Regulus, P. *et al.*, *Rapid. Commun. Mass Spectrom.* **18**, (2004). 2223-2228



Mechanism of formation of dCyd341



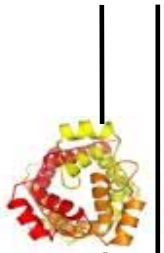
dCyd341 a cluster DNA lesion!



Regulus, P. *et al.*, *PNAS* **104**, (2007). 14032-14037

Nuclear protein

?



DNA-Protein Crosslinks?

Sczepanski, J. T. *et al.*, *PNAS* **107**, (2010). 22475-22480

Importance of sugar oxidation in the genotoxicity of ionizing radiation

Message

The DNA structure strongly influences the mechanisms of decomposition of initially generated radicals

(influence of surrounding bases, proteins…)

and also efficacy of repair of oxidative DNA lesions

There is a need of chemistry in radiation biology…

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Tandem Lesions

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dCyd341

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Thank you for your attention…

9th Winter Research Conference
Les Houches, France, March 11 to 16, 2012

DNA Damage: formation, repair, health consequences and industrial issues

Organized by the laboratory « Lésions des Acides Nucléiques »
CEA-Grenoble, France

http://www.cerlib2012.org/

List of invited speakers

D. Angelov (ENS Lyon, France)
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I. Beerman (Harvard Medical School, USA)
V. Bohr (NIH, Baltimore, USA)
S. Bombard (CNRS, Paris, France)
B. Castaing (CNRS, Orléans, France)
R. Fuchs (CNRS, Marseille, France)
M. E. Geachintov (University of New York, USA)
M. M. Greenberg (Johns Hopkins University, USA)
T. Halazonetis (University of Geneva, Switzerland)
J. Hall (Institut Curie, Orsay, France)
Z. Herceg (IARC / WHO, Lyon, France)
P. Karran (Cancer Research UK, London, GB)
M. Kirsch-Volders (Vrije Universiteit, Brussels, Belgium)
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